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NOTES

Thymus Involution Induced by Mouse Hepatitis Virus A59 in BALB/c Mice

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Mouse hepatitis virus A59 (MHV-A59) infection of adult BALB/c mice induced a severe, transient atrophy of the thymus. The effect was maximal at 1 week after infection, and thymuses returned to normal size by 2 weeks after infection. There was no effect of glucocorticoids, since thymus atrophy was also found in adrenalectomized, infected mice. In infected thymus, immature $CD4^+$ $CD8^+$ lymphocytes were selectively depleted, and apoptosis of lymphocytes was increased. The MHV receptor glycoprotein MHVR was detected on thymus epithelial cells but not on T lymphocytes. In a small number of stromal epithelial cells, but in very few lymphocytes, the viral genome was detectable by in situ hybridization. These observations suggested that MHV-A59-induced thymic atrophy results not from a generalized lytic infection of T lymphocytes but rather from apoptosis of immature double-positive T cells that might be caused by infection of a small proportion of thymus epithelial cells or from inappropriate secretion of some factor, such as a cytokine.

Mouse hepatitis viruses (MHV) cause liver and central nervous system disease and a wide variety of immunological dysfunctions (6, 8, 10, 13, 25, 32, 35, 53, 54, 61). The pathogenesis induced by these viruses depends on both the characteristics of the virus strain and the genetic background of the infected mice (2, 30, 56, 57). Infection of BALB/c mice with MHV-JHM or MHV-A59 or of C57BL/6 mice with MHV-3 is followed by a marked thymus involution (11, 29, 32). In the latter case, thymic lymphocyte decrease follows viral dissemination from infected stromal cells (25, 33). In the present paper, we investigated the mechanisms involved in thymus atrophy observed in BALB/c mice infected with MHV-A59 and whether the distribution of expression of the carcinoembryonic antigen-related glycoproteins that serve as receptor for the virus (MHVR [15, 16, 64–66]) could account for this thymus involution.

The effect of infection on the thymuses of specific-pathogen-free female BALB/c mice, bred by G. Warnier at the Ludwig Institute for Cancer Research, was analyzed at different times after intraperitoneal injection of approximately 10^4 tissue culture infectious doses of MHV-A59 grown in NCTC 1469 cells. MHV-A59 induced a dramatic but transient decrease in thymus size that was most pronounced 1 week after virus inoculation. There was a marked disappearance of lymphocytes, predominantly from the cortical region of the thymuses of infected mice (Fig. 1). Thymus cell counts had dropped by 80 to 90% by day 7 postinfection (p.i.) but returned to normal levels 5 days later (Table 1). Thus, in BALB/c mice infected with MHV-A59, the thymus involutes transiently to the same extent as that reported after infection with MHV-JHM or, in C57BL/6 animals, with MHV-3 (11, 25, 29, 32, 33).

To further evaluate the effect of MHV-A59 infection on the thymus, subsets of immature double-negative $CD4^-$ $CD8^-$ and double-positive $CD4^+$ $CD8^+$ cells, as well as more mature single-positive $CD4^+$ $CD8^-$ and $CD4^-$ $CD8^+$ cells, were analyzed by flow cytometry. After teasing with forceps and lysis of erythrocytes in 0.83% NH_4Cl, the cells were incubated for 60 min at 4°C in HAFA buffer (137 mM NaCl, 5 mM KCl, 0.4 mM $MgSO_4$, 0.3 mM $MgCl_2$, 5 mM glucose, 4 mM $NaHCO_3$, 1 mM EDTA, 1 mM phosphate, 20 mM NaN_3, 100 U of penicillin per ml, 100 μg of streptomycin per ml [pH 7.4], supplemented with 3% fetal calf serum) with fluoresceinated anti-CD8 and biotinylated anti-CD4 monoclonal antibodies (3.168.1 [51] and GK1.5 [14], respectively) (made available by F. W. Fitch and obtained through the courtesy of H. R. MacDonald and P. G. Coulie) and then with streptavidin-RED613 (Gibco BRL, Gaithersburg, Md.). After MHV-A59 infection, the absolute number of double-positive $CD4^+$ $CD8^+$ cells decreased to 1 to 7% of the normal levels, depending on the experiment. In contrast, the absolute number of $CD4^+$ $CD8^-$ cells decreased only moderately, whereas the relative proportion of this subpopulation increased significantly (Table 1). Both the $CD4^-$ $CD8^-$ and the $CD4^-$ $CD8^+$ subsets were not significantly affected by MHV-A59 infection.

Such a selective decrease in the $CD4^+$ $CD8^+$ population may result from apoptosis (41, 55, 58). To test whether programmed cell death was induced by MHV-A59 infection, electron microscopy was performed on thymus tissue that had been minced, fixed in 2.5% glutaraldehyde, and postfixed in 1% OsO_4 solution before embedding in Epon 812 (Fluka). After MHV-A59 infection, numerous cells showed images that are classically associated with apoptosis: nuclear condensation and fragmentation (Fig. 2). No MHV virions were observed in these cells.

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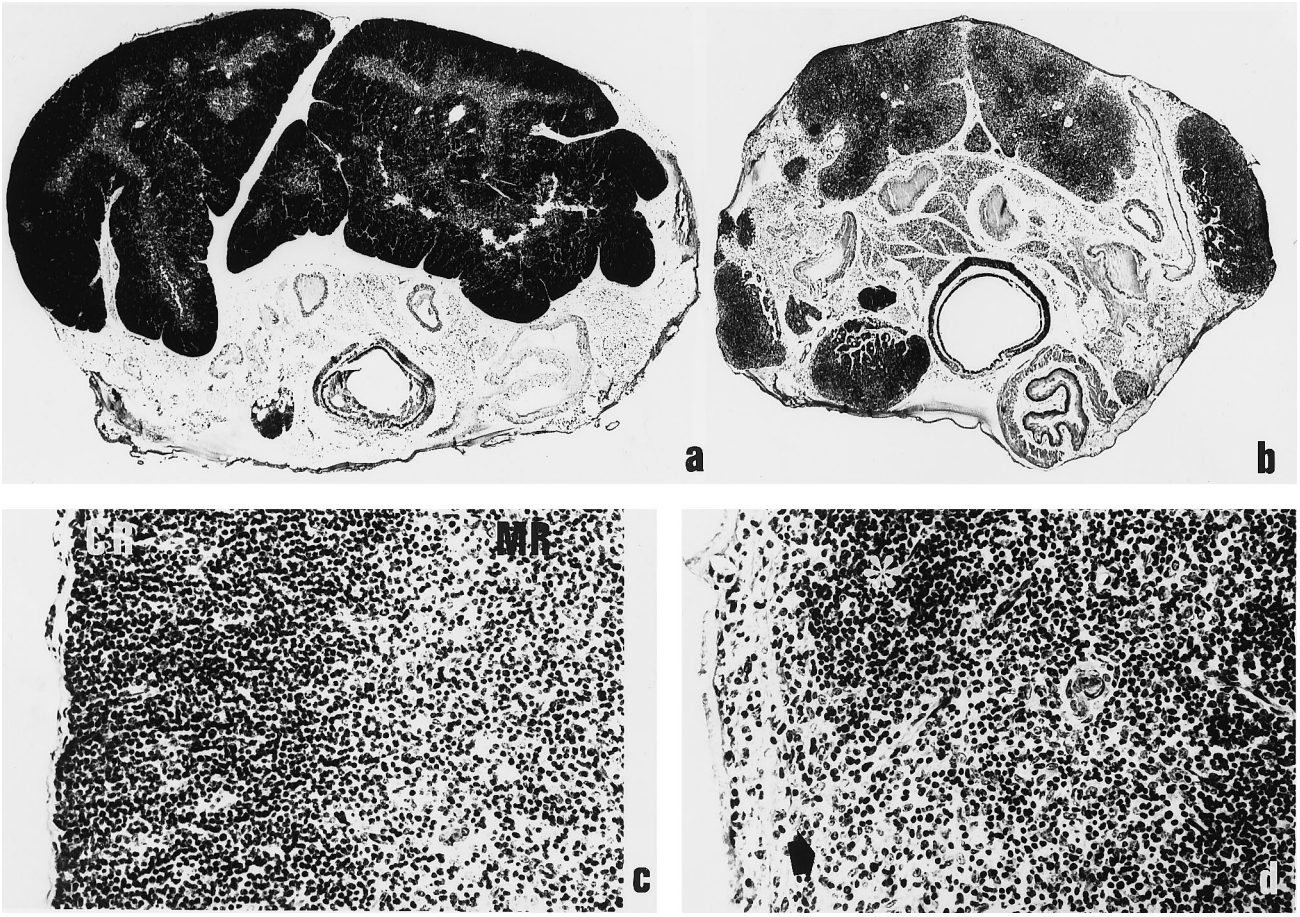

FIG. 1. Thymus involution in BALB/c mice infected with MHV-A59; whole cross-section of a thymus from a control 8- to 12-week-old BALB/c mouse (a) and a mouse 5 days p.i. with MHV-A59 (b). The infected thymus shows a large reduction in size as well as an inverted cortical image. Higher magnification of the control thymus shows the normal cell population in the cortical (CR) and the medullary (MR) regions (c). At the same magnification, the thymus cortical region of the infected animal (d) shows a focal decrease in lymphocytes (arrow), while other zones are less affected (star). Magnifications are ×16 for panels a and b and ×150 for panels c and d.

To confirm that these cells had DNA fragmentation, tissue sections were analyzed by in situ end labelling of fragmented DNA as previously described (63). Briefly, after a 4% paraformaldehyde fixation, thymuses from infected and control mice were paraffin embedded. Sections were treated with *Escherichia coli* DNA polymerase I and deoxynucleoside triphosphates, one of which was biotin labelled. The reaction product was detected with a streptavidin peroxidase that was revealed by a diaminobenzidine reaction. As shown in Fig. 3, only a few apoptotic cells were detected in thymuses from normal uninfected mice. In contrast, 4 days after MHV-A59 infection, the number of apoptotic cells was strongly increased, while the total number of lymphocytes was dramatically decreased. Thus, a much higher proportion of apoptotic cells was found in thymuses from MHV-A59 infected mice than in those from normal animals. This MHV-induced programmed cell death could account for the selective loss of $CD4^+$ $CD8^+$ cells from the thymus observed after virus infection. Apoptosis of T lymphocytes has been reported after infection with other RNA viruses, such as lymphocytic choriomeningitis virus (47), Moloney murine leukemia virus (49), feline immunodeficiency virus (4), and human immunodeficiency virus (39). This mechanism also results in a preferential decrease of $CD4^+$ $CD8^+$ thymic lymphocytes after infection with human immunodeficiency virus (5).

TABLE 1. Thymus lymphocyte subpopulations after MHV-A59 infection

Time p.i. (days)[a]	No. of lymphocytes (10^6)[b]	Lymphocyte subpopulations (%)[c]			
		$CD4^-$ $CD8^-$	$CD4^+$ $CD8^+$	$CD4^+$ $CD8^-$	$CD4^-$ $CD8^+$
0	49 ± 7	3.0	81.7	13.8	1.7
3	29 ± 3	4.7	72.3	17.6	5.7
7	8 ± 2	21.0	37.5	30.5	11.2
12	62 ± 5	6.6	79.4	10.3	3.7

[a] Groups of four BALB/c mice.
[b] Determined for individual mice after teasing of thymus with forceps (means ± standard errors).
[c] Determined by flow cytometry on pooled thymus cells.

Several mechanisms could lead to apoptosis in the course of a viral infection. These included stress-induced glucocorticoid release, direct virus-induced apoptosis, or apoptosis caused by virus-induced release of cytokines. We studied such mechanisms for thymus atrophy induced by MHV-A59 in regard to the cellular distribution of the viral receptor glycoprotein.

Adrenal glucocorticoid secretion may induce programmed cell death leading to thymus involution (21), as reported after

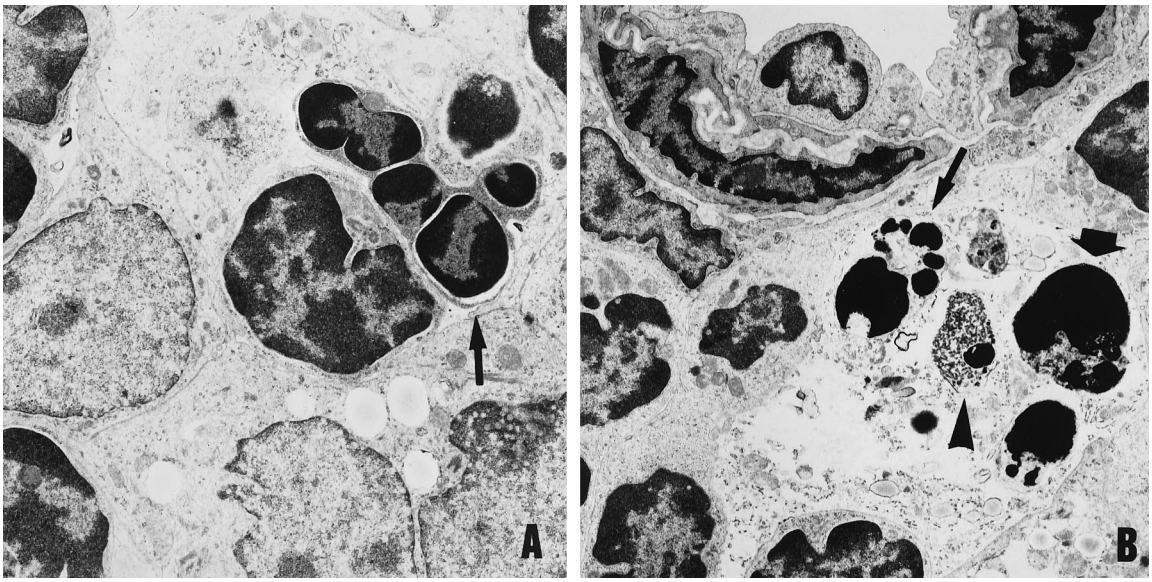

FIG. 2. Electron microscopy of thymuses from BALB/c mice infected with MHV-A59. Thymus tissue from an infected mouse (day 5 p.i.) shows classical features of apoptosis: crown-like chromatin condensation (A [arrow]), which is followed by a complete nuclear condensation (B [large arrow]) and then by nuclear fragmentation (B [arrow]) and finally by disappearance of the cell (B [arrowhead]). Magnification, ×18,000 and ×14,500 for panels A and B, respectively.

infection with lactate dehydrogenase-elevating virus (50). To determine whether such a glucocorticoid release was involved in MHV-induced apoptosis of thymocytes, thymus cells were counted after MHV-A59 infection of adrenalectomized and mock-adrenalectomized BALB/c mice obtained from Iffa Credo (L'Arbresle, France). The efficiency of the adrenalectomy was indicated by accelerated and increased the symptomatology in mice that were deprived of their adrenal glands compared with mock-adrenalectomized animals. Since adrenalectomized animals died by about 6 days postinoculation, thymocytes were counted 5 days after MHV-A59 inoculation. The thymus size was much larger in animals from this source than in mice from the Ludwig Institute for Cancer Research colony. At 5 days p.i., thymus atrophy had not yet reached a maximum; however, a similar decrease in thymocyte counts (between 55 and 65%) was observed in mock-adrenalectomized or adrenal-

FIG. 3. Apoptosis in thymuses from BALB/c mice infected with MHV-A59. In situ end labelling of thymus sections from control (A) and infected (day 4 p.i.) (B) mice. A few labelled cells (arrows) are observed in the control animals, whereas their number is largely increased in the infected mice. Phase interference shows a markedly decreased total cell number in the infected thymus (B). Magnification, ×625.

TABLE 2. Thymus involution in adrenalectomized mice

Treatment	Infection[a]	Thymocyte count[b]
Mock		181 ± 4
Mock	MHV	67 ± 14
Adrenalectomy		191 ± 17
Adrenalectomy	MHV	88 ± 36

[a] MHV-A59 was inoculated intraperitoneally on day 0.
[b] Thymocytes were counted on day 5 p.i. Results are for groups of four to six BALB/c mice (means ± standard errors).

ectomized MHV-infected mice (Table 2). These data suggest that glucocorticoids did not induce the thymocyte apoptosis associated with MHV-A59 infection.

Programmed cell death may directly result from cell infection. Influenza virus and Sindbis virus infection of cell lines in vitro results in programmed cell death of the infected cells (23, 36). Similarly, the VP3 protein of chicken anemia virus induces apoptosis of infected lymphocytes (44), resulting in depletion of the thymic cortex (24). Apoptosis of fibroblasts infected with MHV is indirectly triggered by their interaction with virus-specific cytolytic T lymphocytes or with B lymphocytes (43, 52). Therefore, we tested whether MHV-A59 directly induces programmed cell death of thymus lymphocytes by infecting thymic cells. For this reason, we first wanted to know if thymus could be infected. Therefore, we analyzed the localization of MHVR in the thymuses of control and MHV-A59-infected animals by immunolabelling as previously described (9, 20). Shortly, after paraformaldehyde fixation, the tissue was embedded in graded sucrose solution (59), frozen in liquid nitrogen, and cut at 1 μm on a Reichert-Jung ultracut E microtome with ultracryotomy system FC4D. Immunolabelling was then performed with, as primary antibody, a rabbit polyclonal serum 655 made by immunizing a rabbit with MHVR glycoprotein which had been immunoaffinity purified from Swiss Webster mice with anti-MHVR monoclonal antibody MAb-CC1 recognizing an epitope in the N domain of MHVR (16), but not BGP2 glycoproteins (42). Incubation of sections with this polyclonal antibody or the control serum was then followed by biotinylated anti-rabbit Fab fraction (Boehringer Mannheim, Mannheim, Germany) and streptavidin peroxidase. After incubation with diaminobenzidine, sections were counterstained in a Mayer Hemalun solution.

Immunolabelling of normal, uninfected thymus sections with anti-MHVR antibody 655 revealed expression of receptor antigen in a finely granular pattern predominantly on the capsule and in the cortical regions (Fig. 4a and b). No labelling of thymus was observed with the control normal rabbit serum. Because of the intimate contact between lymphoid and stromal cells in the normal thymus, it was difficult to determine which type of cells was expressing the receptor. Therefore, we took advantage of the depletion of thymic lymphocytes following MHV-A59 infection in order to address this question. Thymus sections of MHV-A59-infected BALB/c mice 6 days p.i. showed that the receptor was expressed on cellular processes and cell bodies that had features typical of thymic epithelial cells, such as pyriform-shaped nuclei with large central nucleoli (Fig. 4c); however, receptor glycoprotein was not detected on thymic lymphocytes by this method. The absence of MHV-A59 receptor expression on thymic T lymphocytes was confirmed by flow cytometry (Fig. 5). To determine whether receptor expression on lymphocytes might be upregulated after infection with MHV-A59, thymus cells were isolated from MHV-A59-infected mice at different times p.i. and analyzed by flow cytometry with anti-MHVR antibody 655. No significant labelling was detected on thymic lymphoid cells from control or MHV-A59-infected mice from 1 to 5 days after virus inoculation (data not shown).

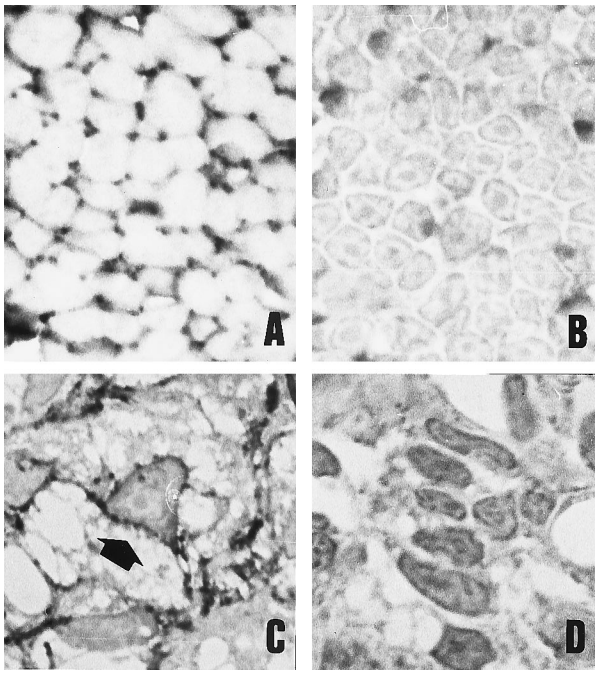

FIG. 4. Expression of MHV-A59 receptor glycoproteins in BALB/c mouse thymus sections. Cryosections of thymus (1 μm thick) from normal BALB/c mice were reacted with anti-MHV receptor antibody 655 (A) or with control rabbit serum (B). Immunolabelling with anti-receptor antibody 655 of thymus sections obtained 6 days after MHV-A59 infection of BALB/c mice demonstrated receptor glycoproteins on cells with cytological features typical of thymic epithelium (C [arrow]), whereas thymic cells incubated with normal rabbit serum were unlabelled (D). Magnification, ×1250.

These results showing that MHV-A59 receptor was expressed on thymus stromal cells but not on T lymphocytes suggest that the former cells are the principal targets for MHV infection in the thymus. However, since it has been shown in vitro that MHV-A59 can spread from infected cells to cells that do not express this receptor (19), T lymphocytes might still be infected after virus inoculation. Therefore, using in situ hybridization, we examined if the presence of MHVR on thymus stromal cells was correlated with MHV-A59 replication in infected BALB/c mice. Thymuses were fixed by immersion either in Bouin fixative or 4% paraformaldehyde and then processed for paraffin embedding. Sections (5 μm thick) were mounted on Superfrost-Plus slides (Menze-Gläser, Brunswick, Germany), deparaffinized, rehydrated, and digested with proteinase K before processing for in situ hybridization. A digoxigenin-labelled antisense oligonucleotide corresponding to a fragment of the MHV-A59 nucleocapsid gene (388^{-} [27]) was diluted at a concentration of 8 pmol/50 μl in a hybridization buffer containing 50% formamide, 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 500 μg of sheared single-strand salmon sperm DNA per ml, 250 μg of yeast tRNA per ml, 1× Denhardt's solution, and 10% dextran sulfate (molecular weight, 500,000). Sense oligonucleotide was used as a control. Hybridization was performed overnight in a humid chamber containing 50% formamide–2× SSC at 37°C. Sections were then rinsed twice in 50% formamide–2× SSC for 10 min at room temperature. They were then slightly fixed with 4% paraformaldehyde and processed for immunolabelling. After

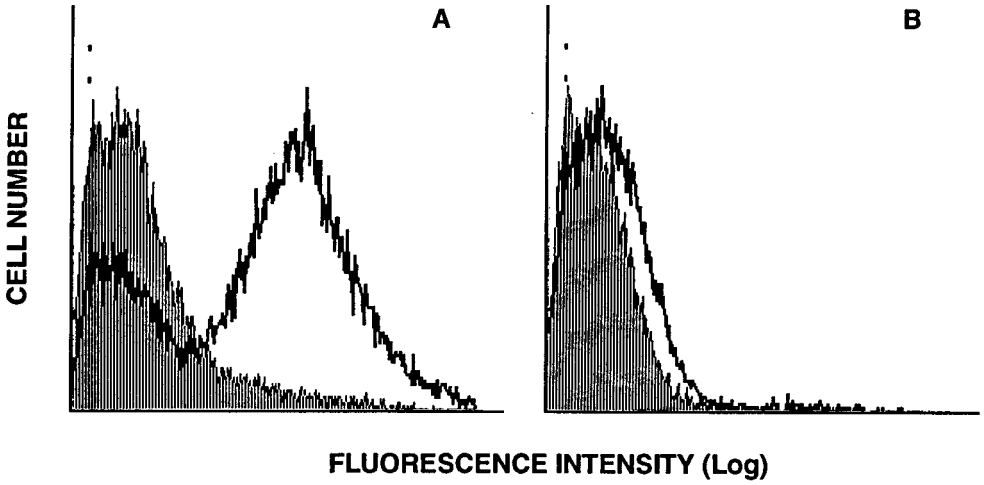

FIG. 5. Flow cytometric analysis of MHVR glycoprotein expression on spleen and thymus cells. Expression of MHVR was analyzed on pooled spleen (A) or thymus (B) cells from three BALB/c mice incubated with normal rabbit serum (hatched zone) or with rabbit anti-MHVR serum 655 (bold line) and then with fluoresceinated goat anti-rabbit antibody.

inhibition of endogenous peroxidase with 0.3% H_2O_2 in deionized water for 30 min, the sections were incubated with 10% bovine serum albumin (BSA) in 0.05 M Tris buffer (pH 7.4). Goat anti-digoxigenin (Boehringer) (1/500 in Tris buffer with 1% BSA) was applied for 90 min. After rinsing in Tris buffer, the sections were incubated with a biotinylated rabbit-anti-goat antibody and then with streptavidin peroxidase. Detection was performed with diaminobenzidine. The sections were slightly counterstained with hematoxylin, dehydrated, and mounted in Eukitt medium.

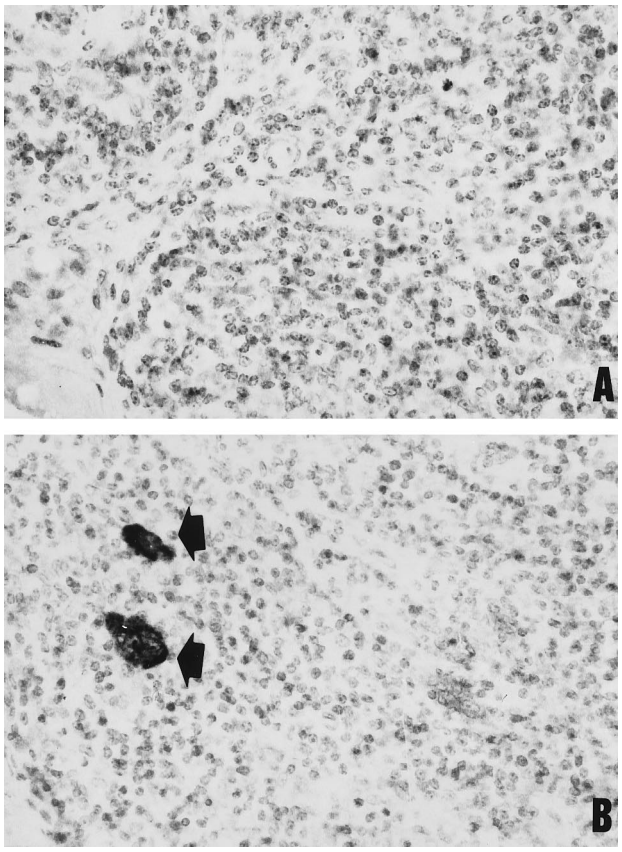

FIG. 6. MHV-A59 replication in thymuses of infected BALB/c mice; in situ hybridization of thymus section from virus-infected BALB/c mice (5 days p.i.) with a labelled antisense probe (B) and a sense probe (A). Rare cells expressing viral mRNA (arrows) were observed in the cortical region (B). These large cells had features compatible with those of thymic epithelial cells. Magnification, ×300.

By in situ hybridization, MHV-A59 RNA was detected only in a very small number of thymus cells (Fig. 6), whereas MHV-A59 RNA was detected in many hepatocytes (data not shown). Most of the infected cells were very large, characteristic epithelial cells, not lymphoid cells. No labelling was observed with the control sense probe in infected animals (Fig. 6) or with the antisense oligonucleotide in thymuses from uninfected animals (data not shown). Because most lymphocytes were not infected, as shown by in situ hybridization and by electron microscopy, virus infection of thymic lymphocytes was not the cause of MHV-A59-induced thymic involution. This relative resistance of thymic lymphocytes to MHV-A59 infection agrees with a previous report (3). In this regard, MHV-A59 differs from MHV-3, which induces nonproductive viral replication in most thymus T lymphocytes in susceptible mouse strains, leading to a decrease of all thymocyte subsets (25, 26, 32, 33).

Apoptosis in the course of viral infection can also result from the secretion of a soluble molecule, either by infected cells or as part of the general response to the infection. Several cytokines, including transforming growth factor β2, have been shown to promote apoptosis of T-cell lines (62). Mouse thymocytes, as well as fibroblastic cells infected with feline immunodeficiency virus, undergo similar active deaths when exposed to tumor necrosis factor alpha (28, 34, 45). Interleukin-4 inhibits thymocyte development in fetal thymus organ cultures (46). On the other hand, several soluble molecules with growth factor or cytokine activity, including insulin-like growth factors, platelet-derived growth factor, interleukin-1, interleukin-3, interleukin-4, interleukin-6, interleukin-7, and interleukin-9, have been reported to protect cells against programmed cell death in some circumstances (1, 22, 37, 40, 48). Since thymic epithelial cells produce such molecules (12, 18), infection of these cells, resulting in lysis or in impairment of their secretory activity, might lead to lymphocyte disappearance, which is suspected to be responsible for the increased susceptibility to

apoptosis found in double-positive lymphocytes after inoculation with murine cytomegalovirus (31).

In summary, our results show that the MHV-A59 receptor glycoprotein, MHVR, was expressed on thymus stromal cells, which are the principal targets for MHV-A59 infection in the thymus, but that MHVR expression was not detectable on thymic T lymphocytes which were relatively resistant to virus infection. We postulate that, in addition to a role in viral pathogenesis, MHVR and related glycoproteins may play important functions in the normal murine immune response (9), since it has been shown in other systems that they promote cell-to-cell adhesion (38, 60). For example, their expression on thymus epithelial cells might mediate homing of immature T lymphocytes to the thymus. Impairment of MHVR-expressing epithelial cells by MHV-A59 infection could then cause abnormal T-cell maturation, leading to apoptosis of immature T lymphocytes and thymus atrophy. This could occur if infection causes stromal cells to elaborate a factor such as a cytokine which induces apoptosis or if infected stromal cells fail to elaborate a trophic factor required for T-cell maturation. Future studies have to focus on the role in the thymus of MHVR and its different isoforms in T-cell maturation and T-cell homing. The rapidly expanding availability of molecular and serological reagents to detect the expression of the many isoforms of the murine carcinoembryonic antigen-related glycoproteins (7, 16, 17, 65) should facilitate the study of the role of these proteins in the immune response as well as in mouse coronavirus infection.

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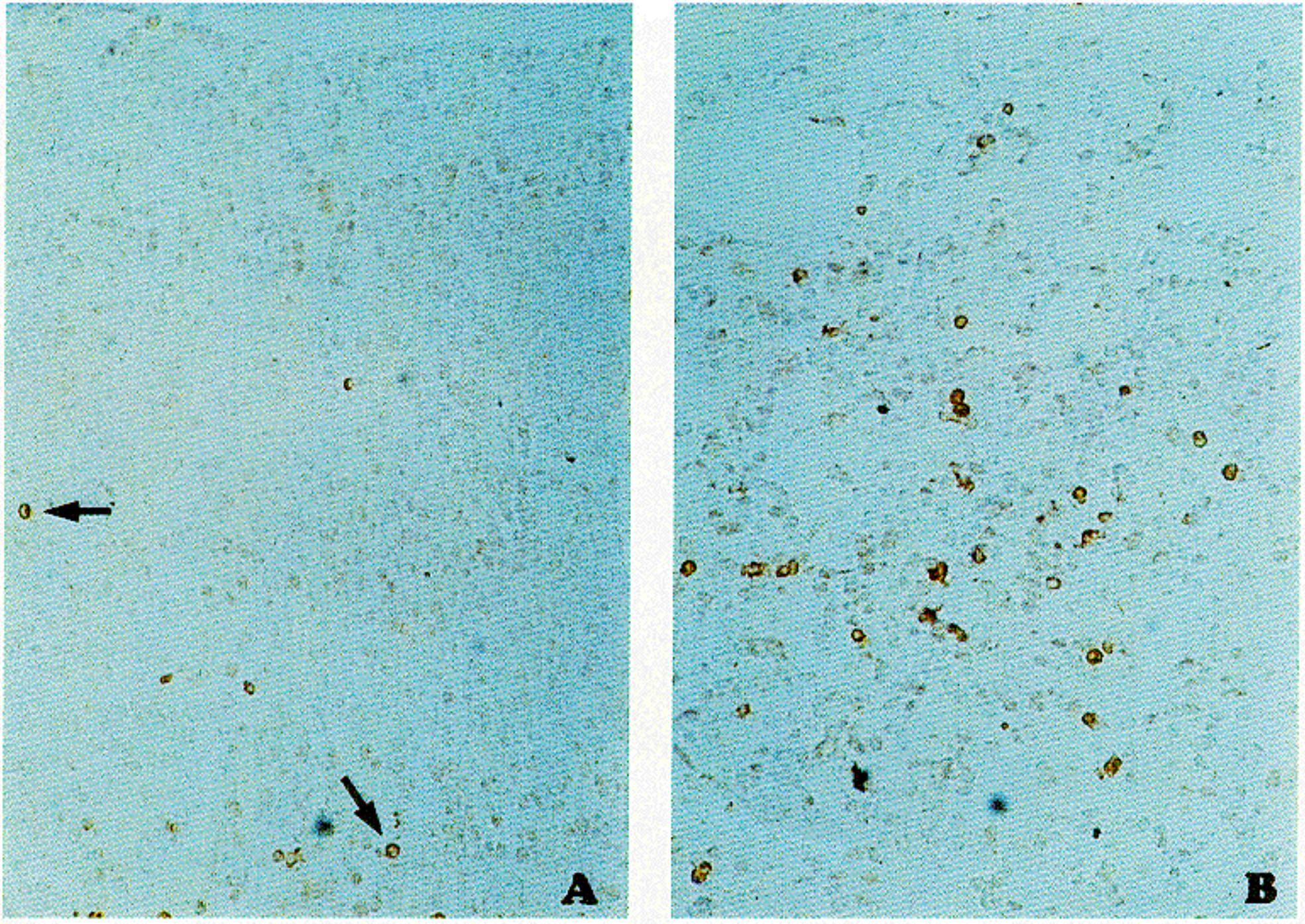
REFERENCES

1. **Ascaso, R., J. Marvel, M. K. L. Collins, and A. Lopez-Rivas.** 1994. Interleukin-3 and bcl-2 cooperatively inhibit etoposide-induced apoptosis in a murine pre-B cell line. Eur. J. Immunol. **24:**537–541.
2. **Bang, F. B., and A. Warwick.** 1960. Mouse macrophages as host cells for the mouse hepatitis virus and the genetic basis of their susceptibility. Proc. Natl. Acad. Sci. USA **46:**1065–1075.
3. **Barthold, S. W., and A. L. Smith.** 1984. Mouse hepatitis virus strain-related patterns of tissue tropism in suckling mice. Arch. Virol. **81:**103–112.
4. **Bishop, S. A., T. J. Gruffydd-Jones, D. A. Harbour, and C. R. Stokes.** 1993. Programmed cell death (apoptosis) as a mechanism of cell death in peripheral blood mononuclear cells from cats infected with feline immunodeficiency virus (FIV). Clin. Exp. Immunol. **93:**65–71.
5. **Bonyhadi, M. L., L. Rabin, S. Salimi, D. A. Brown, J. Kosek, J. M. McCune, and H. Kaneshima.** 1993. HIV induces thymus depletion *in vivo*. Nature (London) **363:**728–732.
6. **Boorman, G. A., M. I. Luster, J. H. Dean, M. L. Campbell, L. A. Lauer, F. A. Talley, R. E. Wilson, and M. J. Collins.** 1982. Peritoneal macrophage alterations caused by naturally occurring mouse hepatitis virus. Am. J. Pathol. **106:**110–117.
7. **Compton, S. R., C. B. Stephensen, S. W. Snyder, D. G. Weismiller, and K. V. Holmes.** 1992. Coronavirus species specificity: murine coronavirus binds to a mouse-specific epitope on its carcinoembryonic antigen-related receptor glycoprotein. J. Virol. **66:**7420–7428.
8. **Cook-Mills, J. M., H. G. Munshi, R. L. Perlman, and D. A. Chambers.** 1992. Mouse hepatitis virus infection suppresses modulation of mouse spleen T-cell activation. Immunology **75:**542–545.
9. **Coutelier, J.-P., C. Godfraind, G. S. Dveksler, M. Wysocka, C. B. Cardellichio, H. Noël, and K. V. Holmes.** 1994. B lymphocyte and macrophage expression of carcinoembryonic antigen-related adhesion molecules that serve as receptors for murine coronavirus. Eur. J. Immunol. **24:**1383–1390.
10. **Coutelier, J.-P., J. T. M. van der Logt, and F. W. A. Heessen.** 1991. IgG subclass distribution of primary and secondary immune responses concomitant with viral infection. J. Immunol. **147:**1383–1386.
11. **Cray, C., M. O. Mateo, and N. H. Altman.** 1993. In vitro and long-term in vivo immune dysfunction after infection of BALB/c mice with mouse hepatitis virus strain A59. Lab. Anim. Sci. **43:**169–174.
12. **Deman, J., C. Humblet, M.-T. Martin, J. Boniver, and M.-P. Defresne.** 1994. Analysis by *in situ* hybridization of cytokine mRNA expression in the murine developing thymus. Int. Immunol. **6:**1613–1619.
13. **de Souza, M. S., A. L. Smith, and K. Bottomly.** 1991. Infection of BALB/cByJ mice with the JHM strain of mouse hepatitis virus alters in vitro splenic T cell proliferation and cytokine production. Lab. Anim. Sci. **41:**99–105.
14. **Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch.** 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. Immunol. Rev. **74:**29–56.
15. **Dveksler, G. S., C. W. Dieffenbach, C. B. Cardellichio, K. McCuaig, M. N. Pensiero, G.-S. Jiang, N. Beauchemin, and K. V. Holmes.** 1993. Several members of the mouse carcinoembryonic antigen-related glycoprotein family are functional receptors for the murine coronavirus mouse hepatitis virus-A59. J. Virol. **67:**1–8.
16. **Dveksler, G. S., M. N. Pensiero, C. B. Cardellichio, R. K. Williams, G.-S. Jiang, K. V. Holmes, and C. W. Dieffenbach.** 1991. Cloning of the mouse hepatitis virus (MHV) receptor: expression in human and hamster cell lines confers susceptibility to MHV. J. Virol. **65:**6881–6891.
17. **Dveksler, G. S., M. N. Pensiero, C. W. Dieffenbach, C. B. Cardellichio, A. A. Basile, P. E. Elia, and K. V. Holmes.** 1993. Mouse hepatitis virus strain-A59 and blocking antireceptor monoclonal antibody bind to the N-terminal domain of cellular receptor. Proc. Natl. Acad. Sci. USA **90:**1716–1720.
18. **Eshel, I., N. Savion, and J. Shoham.** 1990. Analysis of thymic stromal cell subpopulations grown in vitro on extracellular matrix in defined medium. II. Cytokine activities in murine thymic epithelial and mesenchymal cell culture supernatants. J. Immunol. **144:**1563–1570.
19. **Gallagher, T. M., M. J. Buchmeier, and S. Perlman.** 1992. Cell receptor-independent infection by a neurotropic murine coronavirus. Virology **191:**517–522.
20. **Godfraind, C., S. G. Langreth, C. B. Cardellichio, R. Knobler, J.-P. Coutelier, M. Dubois-Dalcq, and K. V. Holmes.** Tissue and cellular distribution of an adhesion molecule in the carcinoembryonic antigen family that serves as a receptor for mouse hepatitis virus. Lab. Invest., in press.
21. **Gruber, J., R. Sgonc, Y. H. Hu, H. Beug, and G. Wick.** 1994. Thymocyte apoptosis induced by elevated endogenous corticosterone levels. Eur. J. Immunol. **24:**1115–1121.
22. **Harrington, E. A., M. R. Bennett, A. Fanidi, and G. I. Evan.** 1994. c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. EMBO J. **13:**3286–3295.
23. **Hinshaw, V. S., C. W. Olsen, N. Dybdahl-Sissoko, and D. Evans.** 1994. Apoptosis: a mechanism of cell killing by influenza A and B viruses. J. Virol. **68:**3667–3673.
24. **Jeurissen, S. H. M., F. Wagenaar, J. M. A. Pol, A. J. van der Eb, and M. H. M. Noteborn.** 1992. Chicken anemia virus causes apoptosis of thymocytes after in vivo infection and of cell lines after in vitro infection. J. Virol. **66:**7383–7388.
25. **Jolicoeur, P., and L. Lamontagne.** 1990. Mouse hepatitis virus 3 pathogenicity and B and T lymphotropisms. Adv. Exp. Med. Biol. **276:**543–552.
26. **Jolicoeur, P., and L. Lamontagne.** 1994. Impaired T and B cell subpopulations involved in a chronic disease induced by mouse hepatitis virus type 3. J. Immunol. **153:**1318–1327.
27. **Jordan, C. A., V. L. Friedrich, C. Godfraind, C. B. Cardellechio, K. V. Holmes, and M. Dubois-Dalcq.** 1989. Expression of viral and myelin gene transcripts in a murine CNS demyelinating disease caused by a coronavirus. Glia **2:**318–329.
28. **Kizaki, H., S. Nakada, Y. Ohnishi, Y. Azuma, Y. Mizuno, and T. Tadakuma.** 1993. Tumour necrosis factor-α enhances cAMP-induced programmed cell death in mouse thymocytes. Cytokine **5:**342–347.
29. **Knobler, R. L., and M. B. A. Oldstone.** 1987. Infection and involution of mouse thymus by MHV-4. Adv. Exp. Med. Biol. **218:**451–453.
30. **Knobler, R. L., L. A. Tunison, and M. B. A. Oldstone.** 1984. Host genetic control of mouse hepatitis virus type 4 (JHM strain) replication. I. Restriction of virus amplification and spread in macrophages from resistant mice. J. Gen. Virol. **65:**1543–1548.
31. **Koga, Y., K. Tanaka, Y.-Y. Lu, M. Oh-Tsu, M. Sasaki, G. Kimura, and K. Nomoto.** 1994. Priming of immature thymocytes to CD3-mediated apoptosis by infection with murine cytomegalovirus. J. Virol. **68:**4322–4328.
32. **Lamontagne, L., J.-P. Descoteaux, and P. Jolicoeur.** 1989. Mouse hepatitis virus 3 replication in T and B lymphocytes correlate with viral pathogenicity. J. Immunol. **142:**4458–4465.
33. **Lamontagne, L., and P. Jolicoeur.** 1991. Mouse hepatitis virus 3-thymic cell interactions correlating with viral pathogenicity. J. Immunol. **146:**3152–3159.

34. **Laster, S. M., J. G. Wood, and L. R. Gooding.** 1988. Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. J. Immunol. **141:** 2629–2634.
35. **Leray, D., C. Dupuy, and J. M. Dupuy.** 1982. Immunopathology of mouse hepatitis virus type 3 infection. IV. MHV3-induced immunodepression. Clin. Immunol. Immunopathol. **23:**539–547.
36. **Levine, B., Q. Huang, J. T. Isaacs, J. C. Reed, D. E. Griffin, and J. M. Hardwick.** 1993. Conversion of lytic to persistent alphavirus infection by the *bcl-2* cellular oncogene. Nature (London) **361:**739–742.
37. **McConkey, D. J., P. Hartzell, S. C. Chow, S. Orrenius, and M. Jondal.** 1990. Interleukin 1 inhibits T cell receptor-mediated apoptosis in immature thymocytes. J. Biol. Chem. **265:**3009–3011.
38. **McCuaig, K., C. Turbide, and N. Beauchemin.** 1992. mmCGM1a: a mouse carcinoembryonic antigen gene family member, generated by alternative splicing, functions as an adhesion molecule. Cell Growth Differ. **3:**165–174.
39. **Meyaard, L., S. A. Otto, R. R. Jonker, M. J. Mijnster, R. P. M. Keet, and F. Miedema.** 1992. Programmed death of T cells in HIV-1 infection. Science **257:**217–219.
40. **Migliorati, G., I. Nicoletti, M. C. Pagliacci, L. D'Adamio, and C. Riccardi.** 1993. Interleukin-4 protects double-negative and CD4 single-positive thymocytes from dexamethasone-induced apoptosis. Blood **81:**1352–1358.
41. **Murphy, K. M., A. B. Heimberger, and D. Y. Loh.** 1990. Induction by antigen of intrathymic apoptosis of $CD4^+$ $CD8^+$ TCR^{lo} thymocytes in vivo. Science **250:**1720–1723.
42. **Nédellec, P., G. S. Dveksler, E. Daniels, C. Turbide, B. Chow, A. A. Basile, K. V. Holmes, and N. Beauchemin.** 1994. *Bgp2*, a new member of the carcinoembryonic antigen-related gene family, encodes an alternative receptor for mouse hepatitis viruses. J. Virol. **68:**4525–4537.
43. **Nishioka, W. K., and R. M. Welsh.** 1993. B cells induce apoptosis via a novel mechanism in fibroblasts infected with mouse hepatitis virus. Nat. Immun. **12:**113–127.
44. **Noteborn, M. H. M., D. Todd, C. A. J. Verschueren, H. W. F. M. de Gauw, W. L. Curran, S. Veldkamp, A. J. Douglas, M. S. McNulty, A. J. van der Eb, and G. Koch.** 1994. A single chicken anemia virus protein induces apoptosis. J. Virol. **68:**346–351.
45. **Ohno, K., T. Nakano, Y. Matsumoto, T. Watari, R. Goitsuka, H. Nakayama, H. Tsujimoto, and A. Hasegawa.** 1993. Apoptosis induced by tumor necrosis factor in cells chronically infected with feline immunodeficiency virus. J. Virol. **67:**2429–2433.
46. **Plum, J., M. De Smedt, G. Leclercq, and B. Tison.** 1990. Inhibitory effect of murine recombinant IL-4 on thymocyte development in fetal thymus organ cultures. J. Immunol. **145:**1066–1073.
47. **Razvi, E. S., and R. M. Welsh.** 1993. Programmed cell death of T lymphocytes during acute viral infection: a mechanism for virus-induced immune deficiency. J. Virol. **67:**5754–5765.
48. **Renauld, J.-C., A. Vink, J. Louahed, and J. Van Snick.** 1995. IL-9 is a major anti-apoptotic factor for thymic lymphomas. Blood **85:**1300–1305.
49. **Saha, K., P. H. Yuen, and P. K. Y. Wong.** 1994. Murine retrovirus-induced depletion of T cells is mediated through activation-induced death by apoptosis. J. Virol. **68:**2735–2740.
50. **Santisteban, G. A., V. Riley, and M. A. Fitzmaurice.** 1972. Thymolytic and adrenal cortical responses to the LDH-elevating virus. Proc. Soc. Exp. Biol. Med. **139:**202–206.
51. **Sarmiento, M., A. L. Glasebrook, and F. W. Fitch.** 1980. IgG or IgM monoclonal antibodies reactive with different determinants on the molecular complex bearing Lyt 2 antigen block T cell-mediated cytolysis in the absence of complement. J. Immunol. **125:**2665–2672.
52. **Shibata, S., S. Kyuwa, S.-K. Lee, Y. Toyoda, and N. Goto.** 1994. Apoptosis induced in mouse hepatitis virus-infected cells by a virus-specific $CD8^+$ cytotoxic T-lymphocyte clone. J. Virol. **68:**7540–7545.
53. **Smith, A. L., K. Bottomly, and D. F. Winograd.** 1987. Altered splenic T cell function of BALB/cByJ mice infected with mouse hepatitis virus or Sendai virus. J. Immunol. **138:**3426–3430.
54. **Smith, A. L., D. F. Winograd, and M. S. de Souza.** 1991. In vitro splenic T cell responses of diverse mouse genotypes after oronasal exposure to mouse hepatitis virus, strain JHM. Lab. Anim. Sci. **41:**106–110.
55. **Smith, C. A., G. T. Williams, R. Kingston, E. J. Jenkinson, and J. J. T. Owen.** 1989. Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. Nature (London) **337:**181–184.
56. **Smith, M. S., R. E. Click, and P. G. W. Plagemann.** 1984. Control of mouse hepatitis virus replication in macrophages by a recessive gene on chromosome 7. J. Immunol. **133:**428–432.
57. **Stohlman, S. A., and J. A. Frelinger.** 1978. Resistance to fatal central nervous system disease by mouse hepatitis virus, strain JHM. I. Genetic analysis. Immunogenetics **6:**277–281.
58. **Swat, W., L. Ignatowicz, H. von Boehmer, and P. Kisielow.** 1991. Clonal deletion of immature $CD4^+8^+$ thymocytes in suspension culture by extrathymic antigen-presenting cells. Nature (London) **351:**150–153.
59. **Tokuyasu, K. T.** 1973. A technique for ultramicrotomy of cell suspension and tissues. J. Cell Biol. **57:**551–565.
60. **Turbide, C., M. Rojas, C. P. Stanners, and N. Beauchemin.** 1991. A mouse carcinoembryonic antigen gene family member is a calcium-dependent cell adhesion molecule. J. Biol. Chem. **266:**309–315.
61. **Virelizier, J.-L., A.-M. Virelizier, and A. C. Allison.** 1976. The role of circulating interferon in the modifications of immune responsiveness by mouse hepatitis virus (MHV-3). J. Immunol. **117:**748–753.
62. **Weller, M., D. B. Constam, U. Malipiero, and A. Fontana.** 1994. Transforming growth factor-beta 2 induces apoptosis of murine T cell clones without down-regulating bcl-2 mRNA expression. Eur. J. Immunol. **24:**1293–1300.
63. **Wijsman, J. H., R. R. Jonker, R. Keijzer, C. J. H. van de Velde, C. J. Cornelisse, and J. H. van Dierendonck.** 1993. A new method to detect apoptosis in paraffin sections: in situ end-labeling of fragmented DNA. J. Histochem. Cytochem. **41:**7–12.
64. **Williams, R. K., G.-S. Jiang, and K. V. Holmes.** 1991. Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins. Proc. Natl. Acad. Sci. USA **88:**5533–5536.
65. **Williams, R. K., G.-S. Jiang, S. W. Snyder, M. F. Frana, and K. V. Holmes.** 1990. Purification of the 110-kilodalton glycoprotein receptor for mouse hepatitis virus (MHV)-A59 from mouse liver and identification of a nonfunctional, homologous protein in MHV-resistant SJL/J mice. J. Virol. **64:** 3817–3823.
66. **Yokomori, K., and M. M. C. Lai.** 1992. Mouse hepatitis virus utilizes two carcinoembryonic antigens as alternative receptors. J. Virol. **66:**6194–6199.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